

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph on page 1 beginning at line 1 with the following rewritten paragraph:

This application is a continuation of Application No. 09/469,211, filed December 22, 1999, which claims foreign priority benefits to Application No. 9828660.2 filed on December 24, 1998 in the United Kingdom, both of which are is incorporated herein by reference in ~~its entirety~~ their entireties.

Please replace the paragraph on page 8 beginning at line 4 with the following rewritten paragraph:

Figure 5 shows a schematic diagram of the plasmid ~~pSK52040~~ pDV35S1 as used in the present invention. The plasmid contains the CaMV 35S promoter, a GUS intron (Vancanneyt *et al.*, 1990) and a CaMV 35S terminator in pBluescript;

Please replace the paragraph on page 8 beginning at line 7 with the following rewritten paragraph:

Figure 6 shows a schematic diagram of the plasmid ~~pSK58040~~ pSK52040 as used in the present invention. The plasmid contains the ohp operator from nucleotide 1036 to nucleotide 1449 of SEQ ID NO: 1 inserted in plasmid pSK52040 into the XhoI site upstream of the CaMV 35S -90 bp core promoter. Downstream of the CaMV 35S core promoter are located a GUS intron and a nos terminator;

Please replace the paragraph on page 8 beginning at line 12 with the following rewritten paragraph:

Figure 7 shows a schematic diagram of plasmid ~~pDV35S1~~ pSK58040 as used in the present invention. The plasmid contains the CaMV 35S promoter and the CaMV 35S terminator in pBluescript;

Please replace the paragraph on page 9 beginning at line 27 with the following rewritten paragraph:

Figure 19 shows a schematic diagram of plasmid pBNP60040 as used in the present invention. The plasmid contains the XhoI/SacI fragment from pSK60040 inserted into the XhoI/SacI sites in pBINplus. This fragment contains the chimeric promoter (~~Seq. ID. No. 13~~ SEQ ID NO: 19), a GUS intron (Vancanneyt *et al.*, 1990) and a nos terminator;

Please replace the paragraph on page 10 beginning at line 34 with the

following rewritten paragraph:

Figure 26 shows a schematic diagram of the plasmid pOH005 as used in the present invention. The plasmid is a double construct in pBINplus, containing the chimeric promoter (~~Seq. ID: 13~~ SEQ ID NO: 19), a GUS intron (Vancanneyt *et al.*, 1990) and a nos terminator, and also containing the ohpR sequence between the CaMV 35S promoter and the nos terminator and also containing the ohpR sequence between the CaMV 35S promoter and the CaMV 35S terminator;

Please replace the paragraph on page 11 beginning at line 4 with the following rewritten paragraph:

Figure 27 shows a schematic diagram of the plasmid pOH006 as used in the present invention. The plasmid is a double construct in pBINplus, containing the chimaeric promoter (~~Seq. ID: 13~~ SEQ ID NO: 19), a GUS intron (Vancanneyt *et al.*, 1990) and a nos terminator, and also containing the translational fusion between the ohpR coding sequence (nucleotide 295 to nucleotide 1035 of SEQ ID NO: ~~4~~ 19) and part of the C1 cDNA (from the NarI at nucleotide 536 to the end of the coding region at nucleotide 839, amino acids 179 to 279 of the C1 protein) between the CaMV 35S promoter and the CaMV 35S terminator;

Please replace the paragraph on page 11 beginning at line 11 with the following rewritten paragraph:

Figure 28 shows a schematic diagram of the plasmid pOH007 as used in the present invention. The plasmid is a double construct in pBINplus containing the chimeric promoter (~~Seq. ID: 4~~ SEQ ID NO: 19), a GUS intron (Vancanneyt *et al.*, 1990) and a nos terminator, and also containing the translational fusion between the ohpR coding sequence (nucleotide 295 to nucleotide 1035 of SEQ ID NO: ~~4~~ 19) and part of the C1 cDNA (from the PstI site at nucleotide 674 to the end of the coding region at nucleotide 839, amino acids 219 to 279 of the C1 protein) between the CaMV 35S promoter and the CaMV 35S terminator.

Please replace the paragraph beginning on page 15 at line 29 and ending on page 16 line 18 with the following rewritten paragraph:

Gene sequence similarity is established by Southern Blot screening. Such screening is initially carried out under low-stringency conditions, which comprise a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (*e.g.* Standard Saline Citrate ('SSC') = 0.15 M sodium chloride; 0.15 M sodium citrate; pH7) concentration. Alternatively, a temperature of about 50°C or less and a

high salt (*e.g.* SSPE= ~~0.280~~ mM 0.280 M sodium chloride; 9 mM disodium hydrogen phosphate; 9 mM sodium dihydrogen phosphate; 1 mM sodium EDTA; pH 7.4). Preferably the screening is carried out at about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5 x SSPE. These conditions will allow the identification of sequences which have a substantial degree of similarity with the probe sequence, without requiring the perfect homology for the identification of a stable hybrid. The phrase ‘substantial similarity’ refers to sequences which share at least 50% overall sequence identity. Preferably, hybridization conditions will be selected which allow the identification of sequences having at least 70% sequence identity with the probe, while discriminating against sequences which have a lower level of sequence identity with respect to the probe. After low stringency hybridization has been used to identify several bacterial whose genome or DNA sub-clones exhibit a substantial degree of similarity with the probe sequence, this subset of genomes or sub-clones is then subjected to higher stringency hybridization, so as to identify those of this subset of genomes or sub-clones having a particularly high level of homology with respect to the probe sequences. Medium stringency conditions comprise a temperature of about 39°C and a medium salt (SSC) concentration. High stringency conditions comprise a temperature of about 42°C or less, and a low salt (SSC) concentration. Alternatively, they may comprise a temperature of 65°C or less, and a low salt (SSPE) concentration. Preferred conditions for such screening comprise a temperature of about 42°C, a formamide concentration of about 20%, and a salt concentration of about 2xSSC, or a temperature of about 65°C, and a salt concentration of about 0.2 SSPE.

Please replace the paragraph on page 17 beginning at line 24 with the following rewritten paragraph:

The expression of the nucleic acid sequence of the second nucleotide sequence (also known herein as the target gene) may be suitably increased or decreased, whether from a basal or ~~medial~~ median level respectively, or completely repressed or activated.

Please replace the paragraph on page 20 beginning at line 32 with the following rewritten paragraph:

The plasmid pSK-59 (Figure 4) was digested with Xho1 and Sall, the 414bp operator region was gel-purified and ligated with pBS52040 (Figure 5 ~~6~~) which had been digested with XhoI and phosphatased. The resulting plasmid was named pSK58040 (Figure ~~6~~ 7).

Please replace the paragraph on page 21 beginning at line 4 with the following rewritten paragraph:

The three oligonucleotides CaMVop2 (SEQ ID NO: 14), CaMVop3 (SEQ ID NO: 15) and CaMVop4 (SEQ ID NO: 16) were annealed in equimolar amounts (500 pmole each primer) and diluted tenfold. 5 μ l of this dilution were used as a template for a PCR reaction (50 μ l total) catalysed by a proof-reading Taq polymerase to generate double stranded product. The PCR product was resolved on an 8% polyacrylamide gel. The 125 bp PCR product was excised and purified using techniques described in Sambrook et al (1989). 1 μ l of the total eluted double stranded DNA solution (50 μ l) was used as a template in a PCR reaction (50 μ l total) primed by oligonucleotide primers CaMVopF1 (SEQ ID NO: 17) and CaMVopR1 (SEQ ID NO: 18) and catalysed by a proof-reading Taq polymerase. The PCR product from this reaction was digested to completion with EcoRV and BamHI and the 133 bp restriction fragment ligated with plasmid pDV35S1 (Figure 7 5) similarly digested to completion with EcoRV and the resulting construct was named pDV60 (Figure 8). The inserted region was sequenced. Plasmid pDV60 (Figure 8) was digested with XhoI and BamHI. The 476bp synthetic promoter restriction fragment (SEQ ID NO: 19) was gel purified as described above and ligated into pSK52040 (Figure 5 6) similarly digested with XhoI and BamHI. This plasmid was named pSK60040 (Figure 9). The chimeric promoter in SEQ ID NO: 48 19 contains a 36 bp region of the ohp operon (from nucleotide 1225 to nucleotide 1260) inserted into the CaMV 35S promoter at nucleotide 21.

Please replace the paragraph on page 22 beginning at line 4 with the following rewritten paragraph:

pDV35S1 (Figure 7 5) was digested with HindIII and SacI and the 668 bp fragment containing the CaMV 35S promoter/terminator was gel-purified and ligated with pUCAP (Figure 12) which was digested with HindIII and SacI. The resulting construct was named pDV35S2 (Figure 13).

Please replace the paragraph on page 22 beginning at line 22 with the following rewritten paragraph:

Plasmid pSK58040 (Figure 6 7) was digested to completion with HindIII and SmaI and the 2837 bp fragment containing the CaMV 35S promoter-GUS-nos terminator was gel-purified and ligated into pBINplus (Figure 17) similarly digested with HindIII and SmaI. The resulting plasmid was named pBNP58040 (Figure 18).